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EXAMINER	
BAUSCH, SARAE L	

ART UNIT	PAPER NUMBER
1634	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)
	10/078,278	WAGNER ET AL.
	Examiner	Art Unit
	Sarae Bausch	1634

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE ____ MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) _____ is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

Supplemental

DETAILED ACTION

1. This action is a supplemental office action. The office action mailed 01/22/2008 has been withdrawn. This supplemental final office action contains the sections of the instant office action in which the declaration was addressed, which was previously omitted in error.

2. Currently, claims 56-68, 70-75 are pending in the instant application. Claims 1-55 and 69 have been canceled. This action is written in response to applicant's correspondence submitted 10/30/2007. All the amendments and arguments have been thoroughly reviewed but were found insufficient to place the instantly examined claims in condition for allowance. The following rejections are either newly presented, as necessitated by amendment, or are reiterated from the previous office action. Any rejections not reiterated in this action have been withdrawn as necessitated by applicant's amendments to the claims. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action. **This action is Final.**

Claim Amendment

3. It is noted that claim 69 does not comply with 37 CFR 1.121, as it contains the text of a canceled claim. In response to this office action, applicant is required to delete the text of claim 69.

Declaration

4. The declaration filed on 10/30/2007 under 37 CFR 1.131 has been considered but is ineffective to overcome claims 56-68 and 70-75 based upon Kigawa in view of Wagner and

claims 56-68 and 70-75 based upon Kigawan in view of Nolan as set forth in the last office action. The declaration is addressed in sections 11 and 13 below.

Maintained Rejections

Claim Rejections - 35 USC § 112- New Matter

5. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claim 56-68, 70-75 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection was previously presented in section 7 of the previous office action mailed 07/26/2006 and is reiterated below.

Newly added claim 56 with the recitation "wherein a positive signal is generated only when two or more components are co-localized, thus allowing detection" is not supported in the specification and raises the issue of new matter. The specification teaches detecting the presence of immobilized probe DNA or RecA bound to MutS wherein the presence of the bound probe or RecA is indicative of the presence of the mutation or SNP in the test DNA (see page 6, lines 22-25) but does not mention a positive signal is generated only when two or more components are co-localized. The specification teaches the if the test DNA sequence is identical to the probe then the test is negative (see page 7, lines 15-17), however the specification does not teach

generating a positive signal. Furthermore the specification teaches the most successful assay formats on page 22, lines 22-30 but does not teach that a positive signal is generated *only* when from two *or more* components are co-localized. As discussed in MPEP 2163.05, section II, the introduction of claim changes which involve narrowing the claims by introducing elements or limitations which are not supported by the as-filed disclosure is a violation of the written description requirement of 35 U.S.C. 112, first paragraph.

Response to Arguments

7. The response traverses the rejection on pages 8-10 of the response mailed 10/30/2007. The response asserts that the examiner has not reviewed the correct specification in maintaining the instant rejection. It is noted that the examiner was erroneous with reviewing the published application and acknowledges that US2004/0024336 A1 refers to application 10/792785. However it is noted that the instant rejection was based on the specification filed on 02/20/2002 in application 10/078278 not application 10/792785. In the last action mailed 06/01/2007, the examiner reviewed the wrong published application in an attempt to respond to applicants response, as it was unclear which citations applicants were referring to in the response.

Applicants points to the following paragraph 46, 69, 101, 113, 114, 118 to provide support for a positive signal is generated only when two or more components are co-localized thus allowing detection (pages 8-9 of the response). The examiner has carefully reviewed these sections of both the instant specification and published application. It is noted that in each of these sections, at most three components are co-localized or simultaneously detected, as such none of these sections provide support for more than three components co-localized.

Applicants assert on page 10, 1st paragraph that the specification clearly teaches that one of the components must be labeled and that subsequent detection is dependent on the co-localization of two or more components. This response has been thoroughly reviewed but not found persuasive. As stated in the response filed 06/30/2007, the specification teaches, at most, simultaneous detection of three labels (see page 17, lines 3-8 and page 19, lines 5-10). The specification does not disclose more than three labels detected simultaneously nor does the specification teach co-localization of two *or more* components. The claims broadly encompass positive signal detection of more than two components, which encompasses 4, 5, 6, etc components detected by a positive signal upon co-localization and the specification does not teach nor describe more than three components detected simultaneously. Furthermore, the claims encompass detecting a positive signal *only* when two or more components are co-localized and the specification does not teach detection of a positive signal with two or more components are co-localized. The specification only describes simultaneous detection of two (or three) labels (see page 19, lines 5-10) and does not teach that detection of a label is a positive signal. Therefore, the specification does not teach co-localization of two "or more" components nor teach that a positive signal is generated "only" when two or more components are co-localized.

The response asserts on page 10, 2nd paragraph, one skilled in the art would realize that a positive signal is generated only when there is co-localization of two or more components of the present invention with one such component being a label. The response asserts that the co-localization of RecA and/or probe DNA with MutS is a feature of the present invention and labeling of one or more of these components allows for detection of such co-localized

components. This response has been thoroughly reviewed but not found persuasive. The specification does not teach a positive signal is generated. The specification does teach that a "a" signal is detected with the presence of both labels or the presence of a third color created by the juxtaposition of the two or three labels. However, the specification does not teach that the signal that is generate *only* when two or more components are co-localized is positive. Furthermore, the specification teaches that the label can be fluorescent and detected by fluorescence measurement (see pg 18, lines 5-9). Detection by fluorescence encompasses both positive and negative signals, for example detection of a fluorescence signal can encompass quenching of the signal, thereby detecting a negative signal. The support in the specification teaches a signal is generated when the presence of two or three components of RecA and MutS; probe DNA and MutS; or RecA, MutS and probe DNA is co-localized (see page 17, lines 3-5 and pg. 19, lines 3-9 or paragraphs 103 and 113 of the published application), however the specification does not provide support for co-localization of two "or more" components nor teach that a positive signal is generated "only" when two or more components are co-localized.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Claim Rejections - 35 USC § 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

9. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

10. Claims 56-68, 70-75 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa in view of Wagner et al. (US Patent 6120992). This rejection was previously presented in section 7 of the office action mailed 06/01/2007 and is reiterated below.

Kigawa et al. teach a method for detecting the presence of a double stranded target nucleic acid sequence using a probe/RecA complex (abstract). Kigawa et al. teach the use of a nucleic acid probe, typically a single stranded nucleic acid prepared by a virus, plasmid, or a cosmid, a probe DNA moiety excised from a vector, or probe from an oligonucleotide synthesizing method (instant claim 58) (see column 5, lines 64-67 and column 6, lines 1-10). Kigawa et al. teach probes with 90-95% homology to the target nucleic acid sequence and a length of 100 to 1500 bases but longer or short polynucleotide probe may be used (instant claim 59) (see column 6, lines 12-18). Further, Kigawa et al. teach nucleotide probes with a label, such as a fluorescent indicator, a radioactive label or a ligand that can be bound to a specific reporter molecule such as biotin and digoxigenin (instant claim 60) (see column 6, lines 23-28). Kigawa et al. teach the use of RecA protein with a detectable label or ligand, such as a fluorescent indicator, a chemiluminscent agent, an enzymatic label, a radioactive label, biotin or digoxigenin

(instant claim 61-62, 65 and 66) (see column 6, lines 61-67). Kigawa et al. teach alternatively detecting the double-stranded target nucleic acid by allowing the probe/RecA complex to react with an anti-RecA antibody with or without a label or ligand (instant claim 64 and 66) (see column 10, lines 50-58). Kigawa et al. teach the hybridization reaction can be performed in the presence of another protein, such as a single-stranded binding protein, if necessary to accelerate the reaction (instant claim 44) (see column 9, lines 18-22). Kigawa et al. teach detecting the presence of the double stranded target sequence by detecting a fluorescent signal derived from the RecA protein having a fluorescent label included in the probe/RecA complex bound to the target sequence detected with a fluorescent microscope or flow cytometer (instant claim 68-69 and 70) (see column 10, lines 24-32). Kigawa et al. teach the use of the probe/RecA hybridization method to detect various types of chromosomal aberration such as deletion and insertion (see column 13, lines 18-21). Kigawa et al. teach the use of the probe/RecA hybridization method to detect a gene containing a genetic defect (see column 13, lines 12-13). Kigawa et al. does not teach the use of MutS protein with RecA for the detection of single nucleotide base pair insertions, deletions or polymorphisms.

Wagner et al. teach the use of an immobilized mismatch binding protein, MutS for detection of mutations, polymorphisms and allele identifications (see column 6, lines 62-67). Wagner et al. teach allele identification by incubating detectably labeled polynucleotides with immobilized mismatch-binding protein and detecting the binding of the heteroduplex to the protein wherein the presence of the labeled polynucleotide bound to the protein is indicative of the presence of the mutation (see column 9, lines 5-38). Wagner et al. teach an immobilized mismatch binding protein can bind and detect a triplex containing a base pair mismatch (see

column 22, lines 53-67). Wagner et al. teach the mismatch binding protein is attached to a solid support directly and indirectly using supports such as nitrocellulose (see column 24, lines 48-55) or avidin-biotin system (see column 25, lines 25-33) (instant claim 60, 62-64, 73-75). Wagner et al. teach genomic DNA amplified by PCR using biotinylated labeled primers (claims 57-58) (see column 59, lines 5-52 and column 53, lines 13-55). Wagner et al. teach either the probe (see column 26, lines 8-67) or the immobilized mismatch binding protein, MutS, can be labeled (see column 24, lines 29-47). Wagner et al. teach the use of MutS allows for powerful discriminatory ability and has the advantage of simplicity, accuracy, ability to be used without radioactivity, and the ability to detect all single base substitutions and mutations (see column 21, lines 10-25).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of detecting the double stranded target nucleic acid using a probe/RecA complex by Kigawa et al. to include the MutS protein detection system of single nucleotide polymorphisms as taught by Wagner et al. to improve the method of probe/RecA detection system by Kigawa et al. The ordinary artisan would have been motivated to improve the method of detecting the double stranded target nucleic acid sequence using the probe/RecA hybridization system by Kigawa et al. with the immobilized mismatch binding protein as taught by Wagner et al. because Wagner et al. teaches that the MutS immobilized detection system provides a powerful discriminatory ability and has the advantage of simplicity, accuracy, ability to be used without radioactivity, and the ability to detect all single base substitutions and mutations. Furthermore, Wagner et al. teaches that the MutS immobilized system can bind and detect a triplex containing a base pair mismatch. Therefore, the ordinary artisan would have had a reasonable expectation of success that the use of MutS could be used in

the be used in the method by Kigawa et al. because Kigawa teaches a method that detect triplex formation of deletions and insertion in a target DNA and Wagner teaches that using immobilized protein binding method allows for detection base pair mismatches, including base pairs in a triplex and teaches the method allows for simplicity and accuracy in of base pair mismatches.

Response to Arguments

11. The response traverses the rejection on pages 6-8 of the response mailed 10/30/2007. The response asserts on page 6, last para. continued to page 7 that the references are silent with respect to generating a positive signal only when two or more components are co-localized. This response has been thoroughly reviewed but not found persuasive. Both Kigawa et al. and Wagner et al. teach generating a positive signal only when two components are co-localized. Specifically, Kigawa et al. teach detection of RecA protein bound to FITC contained in the target nucleic acid/probe complex the presence of the target nucleic acid could be detected by fluorescence microscope (see column 19, lines 44-56). Kigawa teaches co localized of at least two or more components, RecA, target nucleic acid and probe complex. Detection of the FITC labeled RecA by fluorescence only occurs when RecA is bound to the target nucleic acid and probe complex. Furthermore, Kigawa teaches a positive signal was generated by detection of fluorescence. Wagner et al. teach generating a positive signal only when DNA is bound to MutS by binding biotinylated oligonucleotides and DNA to MutS immobilized to a nitrocellulose filter followed by detection of the immobilized biotin by binding horse radish peroxidase conjugated streptavidin and ECL development (See example 1, column 46, lines 30-65 and column 47, lines

15-42). Therefore, Wagner teaches co localization of at least two or more components, MutS, target DNA, and probe, as well as a positive signal when two or more component are co-localized or bound, by binding streptavidin to biotin followed by ECL development and Xray exposure.

The response asserts that Kigawa does not teach the use of RecA for the detection of single nucleotide base pair of anything. The response asserts that Kigawa teaches the detection of ploidy and detection of genetic alterations by means of fluorescent microscopic. The response asserts that detection of a change as taught by Kigawa would require a very large insertion or deletion and single base pair deletion or insertion could never be detected in the system of Kigawa. This response has been thoroughly reviewed but not found persuasive. The examiner is not asserting that Kigawa teaches the detection of single base pair insertions or deletion, however Kigawa provides motivation that the method can detect single base pair insertions, deletions, or changes. Kigawa teaches the use of probes that are *at least* 90 to 95% homologous to the target nucleic acid (see column 6, lines 12-18 and instant claim 59), which encompasses a single base pair change, insertion or deletion depending on the size of the probe. Furthermore, Kigawa teach a gene containing a genetic defect can be a target sequence (See column 13, lines 8-10). Therefore, Kigawa provides motivation that a single base pair insertion, deletion, or polymorphism could be detected in the method of Kigawa, as the probe needs to be at least 90-95% homologous to the target and a gene with a genetic defect can be detected. With regard to applicants assertion that a single base pair deletion or insertion could never be detected by Kigawa, as stated in MPEP 2145 [R-2], "The arguments of counsel cannot take the place of evidence in the record. In re Schulze, 346 F.2d 600, 602, 145 USPQ 716, 718 (CCPA 1965); In

re Geisler, 116 F.3d 1465, 43 USPQ2d 1362 (Fed. Cir. 1997) ("An assertion of what seems to follow from common experience is just attorney argument and not the kind of factual evidence that is required to rebut a prima facie case of obviousness.")." See MPEP § 716.01(c) for examples of attorney statements which are not evidence and which must be supported by an appropriate affidavit or declaration. In the instant case, the attorney's argument that a single base pair deletion or insertion could never be detected by Kigawa is not factual evidence and requires an appropriate affidavit or declaration to be of probative value which includes inoperability of the prior art. As stated above Kigawa teaches the use of probes that are at least 90-95% homologous to the target nucleic acid, therefore Kigawa teaches that base changes of at least one nucleotide can be tolerated in the method by Kigawa.

This should not be construed as an invitation for providing evidence. As further stated in the MPEP 716.01 regarding the timely submission of evidence:

A) Timeliness.
Evidence traversing rejections must be timely or seasonably filed to be entered and entitled to consideration. In re Rothermel, 276 F.2d 393, 125 USPQ 328 (CCPA 1960). Affidavits and declarations submitted under 37 CFR 1.132 and other evidence traversing rejections are considered timely if submitted:
(1) prior to a final rejection,
(2) before appeal in an application not having a final rejection, or
(3) after final rejection and submitted
(i) with a first reply after final rejection for the purpose of overcoming a new ground of rejection or requirement made in the final rejection, or
(ii) with a satisfactory showing under 37 CFR 1.116(b) or 37 CFR 1.195, or
(iii) under 37 CFR 1.129(a).

The response asserts on page 7, 2nd full paragraph, that Kigawa teaches away from co-localized signal generation. The response asserts that the probe and test DNA that form a D loop must have sequence differences that result in formation of mispaired or unpaired bases in a probe/test duplex region. The response asserts that in contrast Kigawa teaches removing part of

the probe/RecA complex that has not been coupled to the double stranded target nucleic acid and as such the combination of Kigawa with Wagner would not yield the instant invention. This response has been thoroughly reviewed but not found persuasive. The passages that applicant is relying upon to teach away from co-localization of signal generation are misplaced. For example, column 9, lines 63-64 teaches washing away any *unreacted* part of probe/RecA complex, which encompasses washing away excess probe/RecA complex that has not bound however the probe/RecA that is bound will remain bound. The washing step does not wash away probe/RecA complex that has been bound. Furthermore, Kigawa teaches probes that are 90-95% homologous to the target nucleic acid which teaches that probes that are not 100% homologous to the target nucleic acid will bind. Additionally, as stated in the first paragraph of this section, Kigawa et al. teach detection of RecA protein bound to FITC contained in the target nucleic acid/probe complex the presence of the target nucleic acid could be detected by fluorescence microscope (see column 19, lines 44-56).

The response asserts on page 7, 3rd paragraph that Wagner teaches that MutS can recognize mismatches in DNA triplexes when there is no such data to support such a suggestion is misplaced. The response asserts that the notion that one kind of triplex suggests that all triplexes can be recognized lacks support in the outstanding action. Applicants suggest that not all duplexes can be recognized. This response has been thoroughly reviewed but not found persuasive. Wagner et al. teaches that MutS can bind and detect triplexes (see column 22, lines 55-60). Wagner teaches the breadth of the ability of MutS to work with multiple different triplex formations and duplex structures. One of ordinary skill in the art would expect that MutS would bind not only the various triplex formations comprising DNA, RNA and PNA as taught in

Wagner but also DNA triplexes without DNA analogues. There is no teaching by Wagner et al. that MutS cannot bind a DNA triplex and therefore there is a reasonable expectation of success that MutS would bind triplex structures. With regard to applicants suggesting that not all duplexes can be recognized, this response has not been found persuasive as the claims are drawn to the addition of MutS either to a triplex or quadruplex structure and do not encompass duplexes being recognized by MutS. With regard to applicants asserts that in vivo data suggest that MutS does not bind RNA duplexes containing mispaired or unpaired bases, this response is not found persuasive as the claims are not drawn to detection of RNA duplexes but to detection of DNA triplexes and Wagner et al. teaches MutS binds to DNA triplexes.

With regard to the declaration under 1.132 filed on 10/30/2007 by Robert Wagner, the declaration is not commensurate in scope with the pending claims. The declaration asserts in vivo and in vitro experiments results confirmed MutS does not recognized mismatches in RNA/DNA duplexes (see para 3-4). Furthermore the declaration asserts that the ability of MutS protein to bind nucleotide mismatch is complex and not all nucleotide duplex structures are recognized by MutS. This declaration has been thoroughly reviewed and has been found insufficient to overcome the instant rejection. As stated above the declaration is not commensurate in scope with the pending claims. The pending claims require MutS to recognize either a triplex or quadraplex structure and do not require the recognition of MutS to bind a duplex. As such the assertion that MutS binding duplexes is complex and not all duplexes will bind is not relevant to the instant pending claims.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

12. Claims 56-68, 70-75 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kigawa et al (US Patent 5965361 Oct 1999) in view of Nolan et al. (WO 99/22029 May 1999). This rejection was previously presented in section 11 of the office action mailed 07/26/2006 and is reiterated below.

Kigawa et al. teach a method for detecting the presence of a double stranded target nucleic acid sequence using a probe/RecA complex (abstract). Kigawa et al. teach the use of a nucleic acid probe, typically a single stranded nucleic acid prepared by a virus, plasmid, or a cosmid, a probe DNA moiety excised from a vector, or probe from an oligonucleotide synthesizing method (instant claim 58) (see column 5, lines 64-67 and column 6, lines 1-10). Kigawa et al. teach probes with 90-95% homology to the target nucleic acid sequence and a length of 100 to 1500 bases but longer or short polynucleotide probe may be used (instant claim 59) (see column 6, lines 12-18). Further, Kigawa et al. teach nucleotide probes with a label, such as a fluorescent indicator, a radioactive label or a ligand that can be bound to a specific reporter molecule such as biotin and digoxigenin (instant claim 60) (see column 6, lines 23-28). Kigawa et al. teach the use of RecA protein with a detectable label or ligand, such as a fluorescent indicator, a chemiluminscent agent, an enzymatic label, a radioactive label, biotin or digoxigenin (instant claim 61-62, 65 and 66) (see column 6, lines 61-67). Kigawa et al. teach alternatively detecting the double-stranded target nucleic acid by allowing the probe/RecA complex to react with an anti-RecA antibody with or without a label or ligand (instant claim 64 and 66) (see column 10, lines 50-58). Kigawa et al. teach the hybridization reaction can be performed in the presence of another protein, such as a single-stranded binding protein, if necessary to accelerate

the reaction (instant claim 44) (see column 9, lines 18-22). Kigawa et al. teach detecting the presence of the double stranded target sequence by detecting a fluorescent signal derived from the RecA protein having a fluorescent label included in the probe/RecA complex bound to the target sequence detected with a fluorescent microscope or flow cytometer (instant claim 68-69 and 70) (see column 10, lines 24-32). Kigawa et al. teach the use of the probe/RecA hybridization method to detect various types of chromosomal aberration such as deletion and insertion (see column 13, lines 18-21). Kigawa et al. does not teach the use of MutS protein with RecA for the detection of single base pair insertions or deletions.

Nolan et al. teach a method of detection of DNA polymorphisms including nucleotide polymorphisms, insertions, and deletions (page 1, line 6-7) that includes using an immobilized mismatch-binding protein-coated microspheres to bind fluorescently labeled, mismatch-containing DNA by flow cytometry (instant claims 68-69) (page 4, lines 24-26). Nolan et al. teach genomic DNA amplified by PCR using fluorescently labeled nucleotide triphosphates (instant claim 57-58 and 71) (page 4, lines 26-28). Nolan et al. teach microspheres bearing immobilized mismatch-binding protein and further teach mismatch binding proteins to include bacterial mismatch-binding protein, MutS, or any other protein that recognizes DNA base pair mismatches which can be immobilized on microspheres by physical absorption or by the use of an affinity tag which binds to an affinity partner immobilized on microspheres, such as biotin affinity tag and avidin/streptavidin binding partner (instant claim 60, 62-64, 73-75) (page 5, lines 23-29 and page 6 Table).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to improve the method of detecting the double stranded target

nucleic acid using a probe/RecA complex by Kigawa et al. to include the MutS protein detection system as taught by Nolan et al. to improve the method of probe/RecA detection system by Kigawa et al. The ordinary artisan would have been motivated to improve the method of detecting the double stranded target nucleic acid sequence using the probe/RecA hybridization system by Kigawa et al. with the mismatch binding protein, MutS immobilized to microspheres taught by Nolan et al. because Nolan et al. teaches that the MutS immobilized detection system provides a high throughput, small volume, and washless method for detecting SNPs in DNA (page 4, lines 5-6). Further, the method of Nolan et al. allows for rapid scanning of mismatch DNA which would improve the detection of RecA/probe complex formation taught by Kigawa et al. The ordinary artisan would have had a reasonable expectation of success that the use of MutS could be used in the method by Kigawa et al. because Nolan et al. teach that the use of MutS immobilized onto microspheres for the detection of SNPs with flow cytometry provides multiparameter detection with excellent sensitivity in a homogenous assay format and multicolor fluorescent detection can be exploited for the simultaneous detection of dozens, or potentially hundred of analytes in a single sample (page 3, lines 9-14).

Response to Arguments

13. The response traverses the rejection on pages 10-11 of the response mailed 10/30/2007. The response asserts that claim 56 requires generation of a positive signal only when two or more components are co-localized thus allowing detecting without removal of unreacted probes. The response asserts that the references cited are silent with respect to generating a positive signal only when two or more components are co-localized. This response has been thoroughly reviewed but not found persuasive. It is noted that the claims do not require the limitation that

detection occurs without removal of unreacted probes. The claims merely require that a positive signal is generated only when two or more components are co-localized. As stated above, Kigawa et al. teaches detection of RecA protein bound to FITC contained in the target nucleic acid/probe complex the presence of the target nucleic acid could be detected by fluorescence microscope (see column 19, lines 44-56). Therefore, Kigawa teaches a positive signal, fluorescence detection, is generated only when RecA, target nucleic acid and probe complex are co-localized.

The response asserts that Kigawa teaches away from co-localization signal generation. The response asserts that Kigawa teaches removing part of the probe/RecA complex that has not been coupled to the double stranded target nucleic acid sequence. This response has been thoroughly reviewed but not found persuasive. The passages that applicant is relying upon to teach away from co-localization of signal generation are misplaced. For example, column 9, lines 63-64 teaches washing away any *unreacted* part of probe/RecA complex, which encompasses washing away excess probe/RecA complex that has not bound but includes maintaining the probe/RecA bound to the DNA. Furthermore, the washing step does not wash away probe/RecA complex that has been bound. Additionally, as stated in the first paragraph of this section, Kigawa et al. teach detection of RecA protein bound to FITC contained in the target nucleic acid/probe complex the presence of the target nucleic acid could be detected by fluorescence microscope (see column 19, lines 44-56), which demonstrates co-localization of two or more components and the generation of a positive signal.

Applicants assert on page 11, 1st paragraph, that Kigawa teaches it is undesirable to use a wash that contains proteolytic enzymes or protein denaturing solutions as not to remove RecA

from the d-loop and submit that in all other known application of RecA where RecA remains bound to the d-loop further enzymatic action on that DNA is inhibited, such as restriction endonuclease, DNA methylase, etc. The response asserts that there is every reason to expect that MutS would not be able to recognize mismatches in a RecA coated d-loop as taught by Kigawa. This response has been thoroughly reviewed but not found persuasive. It is unclear what applicant is asserting in this section. It is well known in the art that proteolytic enzymes and protein denaturing solutions will remove the RecA from the d-loop and this passage of Kigawa is teaching that these components, proteolytic enzymes which are enzymes that act on proteins, in a wash solution will dissolve RecA, the removal of RecA is not desirable in the method of Kigawa, nor in the instant claimed invention. In both the method of Kigawa and applicants method the addition of proteolytic enzymes or protein denaturing solutions will dissolve RecA from the d-loop. Additionally, the assertion that restriction endonucleases and DNA methylase are inhibited when RecA is bound is perplexing as Kigawa is not teaching that DNA methylase or restriction endonucleases are not inhibited in the RecA complex nor is Kigawa teaching that these components (DNA methylase or restriction endonucleases) will dissolve RecA. Kigawa is teaching that enzymes that act on proteins will dissolve RecA, however Kigawa does not teach that enzymes that act on DNA will act on DNA when bound to RecA. With regard to applicants assertion that there was no expectation of success that MutS would a RecA coated d-loop, it is noted that the claims are not limited to MutS recognizing a RecA coated d-loop, the claims broadly encompass MutS binding to one or more base pair mismatches present in the three stranded or four stranded portion of the DNA and detecting the presence of MutS bound to the DNA structure and the prior art teaches that MutS can recognize a triplex (see Wagner et al. US

Patent 610992, , column 22, lines 55-62) and Kigawa teaches the addition of other proteins to the RecA coated d-loop can be used to accelerate the reaction (See column 8, lines 34-39) therefore there is teaching in Kigawa that there is a reasonable expectation of success that other components, such as proteins, can be used in the method. Furthermore based on the teaching of Nolan that MutS can bind and stabilize mismatches, there is an expectation that MutS can recognize a RecA coated d-loop comprising mismatches.

The response asserts on page 11, 2nd paragraph that MutS is not a single stranded binding protein which requires double stranded DNA. The response asserts that therefore Kigawa fails to teach or suggest the instant claims. The response asserts that even if one were to combine Kigawa with Nolan the combination would not yield the instant invention. This response has been thoroughly reviewed but not found persuasive. It is noted that the examiner was not asserting that MutS is a single stranded binding protein. The teachings that additional proteins can be added to RecA coated complex, as taught by Kigawa using a single stranded binding proteins to accelerate the reaction, provides motivation and reasonable expectation of success that additional proteins can be added to the method of Kigawa, including mismatch stabilizing protein, as Kigawa teaches the use of probes coated with RecA that are 90-95% homologous to the target DNA, which encompass mismatches. Based on the teaching in the prior art, one of ordinary skill in the art would have been motivated to use MutS with RecA for detection of mutations as '992 teaches that MutS binds triplexes, Kigawa teaches the use of additional proteins to accelerate the RecA triplex formation and coupled with the teaching of Nolan that the use of MutS provides for a rapid scanning, high throughput method for detection of mismatches.. Therefore, based on the teachings of Kigawa in view of Nolan, coupled with the evidence in the

art with the teaching that MutS binds a triplex, one of skill in the art would have been motivated to use MutS with RecA to detection deletions, insertions, and SNP in a target nucleic acid. With regard to applicants asserts that the combination of Kigawa and Nolan would not yield the instant invention, this response has been thoroughly reviewed and not found persuasive as the combination of Kigawa in view of Nolan would yield a method of detecting the double stranded target nucleic acid using a probe/RecA complex as taught by Kigawa et al. with the MutS protein detection system as taught by Nolan et al. as discussed in rejection above.

With regard to the declaration filed on 10/30/2007 by Robert Wagner, the declaration states on page 3, paragraph 5, that it was understood that RecA bound to a d-loop structure and inhibited enzymatic action on that DNA structure. The declaration provides evidence to support this assertions. This declaration has been thoroughly reviewed but not found persuasive to overcome the rejection of record because the facts presented are not germane to the rejection at issue. The examiner agrees with the assertions in the declaration that RecA bound d-loop structures inhibit enzymatic action on DNA structures. It is noted however that none of the references relied upon in this rejection teach that the RecA bound d loop structure is not inhibited by enzymatic actions on the DNA structure. The reference by Kigawa does teach that enzymatic actions on proteins will dissolve the RecA bound d loop, however the declaration does not address proteolytic enzymes with RecA bound to a d loop. Therefore, the declaration is insufficient to overcome the rejection.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

14. No claims are allowable.

15. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sarae Bausch whose telephone number is (571) 272-2912. The examiner can normally be reached on M-F 9am-5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax phone number for the organization where this application or proceeding is assigned is (571) 273-8300.

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